Leading Edge
Review
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Mechanisms and Functions of Inflammasomes

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Recent studies have offered a glimpse into the sophisticated mechanisms by which inflammasomes respond to danger and promote secretion of interleukin (IL)-1β and IL-18. Activation of caspases 1 and 11 in canonical and noncanonical inflammasomes, respectively, also protects against infection by triggering pyroptosis, a proinflammatory and lytic mode of cell death. The therapeutic potential of inhibiting these proinflammatory caspases in infectious and autoimmune diseases is raised by the successful deployment of anti-IL-1 therapies to control autoinflammatory diseases associated with aberrant inflammasome signaling. This Review summarizes recent insights into inflammasome biology and discusses the questions that remain in the field.

Introduction

From the primitive lamprey to humans, vertebrates use innate and adaptive immune systems to defend against pathogens (Boehm et al., 2012). In mammals, the innate immune system mounts the initial response to threats. Concomitantly, dendritic cells and other antigen-presenting cells (APCs) relay information about the harmful agent to B and T lymphocytes of the adaptive immune system. Lymphocytes have diverse antigen receptors, and clonal expansion of the cells that recognize the foreign material culminates in its targeted removal (Koch and Radtke, 2011). Antigen receptor gene rearrangements in lymphocytes enable the adaptive immune system to recognize seemingly any antigen, but innate immune cells detect pathogens with a fixed number of germline-encoded "pattern recognition receptors" (PRRs) (Takeuchi and Akira, 2010). PRRs detect unique microbial structures termed pathogen-associated molecular patterns (PAMPs). Microbial nucleic acids, bacterial secretion systems, and components of the microbial cell wall are examples of the conserved microbial factors that are sensed by PRRs. Damaged host cells can also trigger PRRs by releasing danger-associated molecular patterns (DAMPs) such as uric acid crystals, ATP, high-mobility group box 1 (HMGB1), and the heat-shock proteins hsp70 and hsp90.

PRRs can be subdivided into two major classes based on their subcellular localization. Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) are transmembrane proteins found in the plasma membrane and endosomes, where they can survey PAMPs and DAMPs in the extracellular milieu. A second class of PRRs resides in intracellular compartments and includes the RIG-I-like receptor (RLR), the AIM2-like receptor (ALR), and the nucleotide-binding domain and leucine-rich repeat-containing (NLR) proteins (Takeuchi and Akira, 2010). We would add to this second class of PRRs the proteins that sense cytosolic DNA and trigger the production of type I interferon (Paludan and Bowie, 2013).

Many PRRs encountering PAMPs and DAMPs trigger signaling cascades that promote gene transcription by nuclear factor-κB (NF-κB), activator protein 1 (AP1), and interferon regulatory factors (IRFs). Target genes encode cytokines, interferons, and other proinflammatory or microbicidal proteins (Takeuchi and Akira, 2010). A subset of NLRs and ALRs triggers a distinct defense mechanism. These proteins assemble cytosolic protein complexes called inflammasomes to activate proinflammatory caspases 1 and 11 (Kayagaki et al., 2011; Martinon et al., 2002). Rapid conversion of procaspase zymogens into enzymatically active proteases results in: (1) the production of proinflammatory IL-1β and IL-18 and (2) the death of the cell.

Here, we review recent progress in our understanding of inflammasome signaling, the consequences of aberrant inflammasome signaling in human disease, and the therapeutic potential of inflammasome modulation in inflammatory diseases.

Canonical and Noncanonical Inflammasomes: Platforms for Caspase-1 and -11 Activation

Canonical inflammasomes convert procaspase-1 into the catalytically active enzyme, whereas an undefined noncanonical inflammasome promotes activation of procaspase-11 (Figure 1). Caspases 1 and 11 belong to a family of aspartate-specific cysteine proteases conserved through evolution. Like caspases 8, 9, and 10, which initiate apoptotic cell death, caspases 1 and 11 have large prodomains that mediate interactions with other proteins. Interaction motifs belonging to the "death domain" superfamily bring the zymogens into the activating protein complex (Lamkanfi and Dixit, 2012; Riedl and Salvesen, 2007). These homotypic interaction domains typically consist of six or seven antiparallel α helices, the relative orientation of which determines their classification as a caspase activation and recruitment

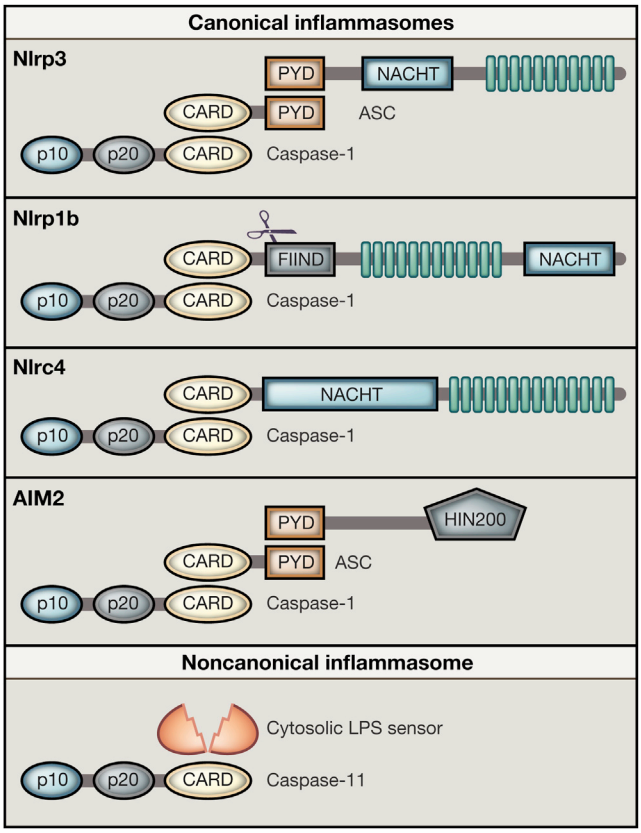

Figure 1. Composition of Canonical and Noncanonical Inflammasomes

The murine NLRs Nlrp3, Nlrp1b, and Nlrc4 and the ALR member absent in melanoma 2 (AIM2) assemble canonical inflammasomes that promote activation of the cysteine protease caspase-1. NLRs are characterized by the combined presence of a NACHT domain and a variable number of LRRs. Most NLRs further contain either a CARD or PYD motif in their amino-terminus. AIM2 is composed of an amino-terminal PYD and a carboxy-terminal DNA-binding HIN200 domain. Note that murine Nlrp1b lacks the amino-terminal PYD motif found in human NLRP1 and is autocatalytically cleaved in its central FIIND domain. Nlrp1b and Nlrc4 recruit caspase-1 via their CARD motifs. The bipartite PYD-CARD adaptor protein ASC may stabilize these interactions and is required for assembly of the AIM2 and Nlrp3 inflammasomes. A currently unknown cytosolic LPS sensor mediates activation of caspase-11 in the noncanonical inflammasome. CARD, caspase recruitment domain; FIIND, domain with function to find; LRR, leucine-rich repeat; NACHT, nucleotide-binding and oligomerization domain; NLR, Nod-like receptor; PYD, pyrin.

domain (CARD), pyrin domain (PYD), death domain (DD), or death effector domain (DED).

The structural rearrangements that occur when procaspases 1 and 11 are activated have not been characterized in detail, but activation is thought to mirror the proximity-induced activation of caspases 8 and 9 (Riedl and Salvesen, 2007). Activation of caspase-9 within the "apoptosome" has several features in common with activation of caspase-1 within canonical inflammasomes. First, the apoptosome and canonical inflammasomes are large, cytosolic, multiprotein complexes that recruit and promote the activation of a CARD-containing initiator caspase. Second, each complex consumes ATP and may have a double-ringed wheel structure with 7- or 8-fold symmetry (Duncan et al., 2007; Faustin et al., 2007; Riedl and Salvesen, 2007). Third, recruitment of procaspases 1 and 9 to their respective complexes is sufficient for them to acquire enzymatic activity (Broz et al., 2010a; Riedl and Salvesen, 2007; Van Opdenbosch et al., 2014), although their maturation into heterotetramers by autoprocessing may lock the proteases into an enzymatically active state. Finally, the intracellular K^+ concentration appears to set the threshold for assembly of the apoptosome and several inflammasomes (Arlehamn et al., 2010; Cain et al., 2001; Muñoz-Planillo et al., 2013). It is possible that cellular stress or damage associated with K^+ efflux relieves a checkpoint safeguarding the cell against unwarranted activation of these lethal caspases.

Caspases 1 and 11 in Immunity and Host Defense

Inflammasomes modulate host defense responses through the production of eicosanoids (von Moltke et al., 2012) and other mechanisms (Lamkanfi, 2011), but the induction of pyroptosis and secretion of proinflammatory IL-1β and IL-18 are considered the prominent outcomes of inflammasome signaling. Pyroptosis is a nonhomeostatic and lytic mode of cell death that requires the enzymatic activity of caspase-1 or -11 (Kayagaki et al., 2011). Cells dying by pyroptosis exhibit cytoplasmic swelling and rupture of the plasma membrane, features that are shared with caspase-independent necroptotic cell death (Lamkanfi, 2011). The molecular events underlying these changes remain obscure, but the application of sophisticated proteomic approaches may illuminate these phenomena in the future. Despite such gaps in our knowledge, pyroptosis has emerged as a key defense against microbial infections (Aachoui et al., 2013; Case et al., 2013; Casson et al., 2013; Kayagaki et al., 2011; Miao et al., 2010a). It is thought to halt the replication of intracellular pathogens by eliminating infected immune cells while simultaneously promoting destruction of surviving bacteria by exposing them to circulating phagocytes and neutrophils. Moreover, pyroptosis may influence adaptive immunity against the infectious agent by releasing antigens into the extracellular milieu, with DAMPs such as IL-1α and HMGB1 acting as potential adjuvants. Further characterization of the in vivo roles of pyroptosis will depend on the identification of markers that are specific for this type of cell death. Biomarkers may also unveil potential differences between caspase-1- versus caspase-11-induced pyroptosis.

Caspases 1 and 11 both induce pyroptosis, but only caspase-1 processes IL-1β and IL-18 (Figure 2). IL-1β is a pyrogenic cytokine that also promotes adaptive T helper 1 (Th_1), Th_{17}, and humoral immunity. IL-18 is important for IL-17 expression by Th_{17} cells and may polarize T cells toward Th_1 or Th_2 profiles in combination with other cytokines (Dinarello, 2009). Unlike most cytokines, IL-1β and IL-18 are not secreted through the classical endoplasmic reticulum-Golgi route but are produced as biologically inactive precursor proteins that are cleaved prior to their secretion as bioactive cytokines (Lamkanfi, 2011). Pro-IL-18 is expressed constitutively in macrophages, whereas expression of pro-IL-1β is regulated by NF-κB-mediated transcription.

Although caspase-11 is required for macrophages to secrete IL-1β and IL-18 after infection with *Escherichia coli*, *Citrobacter rodentium*, or *Vibrio cholerae*, caspase-1 must be activated too (Gurung et al., 2012; Kayagaki et al., 2011). In contrast, pyroptosis in response to these bacteria requires caspase-11, but not

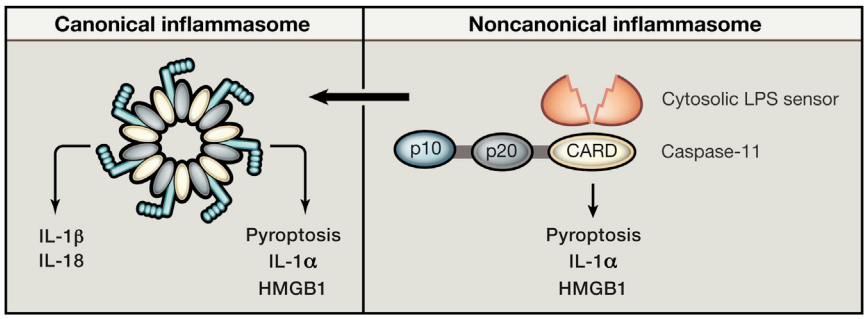

Figure 2. Major Effector Mechanisms of the Canonical and Noncanonical Inflammasomes

Assembly of the canonical inflammasome complexes promotes the proximity-induced autoactivation of caspase-1. Caspase-1 subsequently converts its substrates proIL-1β and proIL-18 into the secreted bioactive cytokines and triggers pyroptosis through an unknown mechanism. A hypothetical noncanonical inflammasome that is activated in response to the detection of cytosolic LPS triggers activation of caspase-11 in macrophages infected with *Escherichia coli*, *Citrobacter rodentium*, *Vibrio cholera*, and other Gram-negative bacteria that enter the cytosol. Caspase-11 mediates pyroptosis and extracellular release of IL-1α and HMGB1 directly and induces secretion of mature IL-1β and IL-18 indirectly through engagement of the canonical Nlrp3 inflammasome.

caspase-1 (Figure 2). Further evidence of a unique role for caspase-11 is provided by the resistance of caspase-11-deficient mice, but not caspase-1-deficient mice, in a model of endotoxic shock (Kayagaki et al., 2011; Wang et al., 1998). Like caspase-1 deficiency, combined loss of the caspase-1 substrates IL-1β and IL-18 does not provide significant protection in this model of endotoxic shock (Kayagaki et al., 2011; Lamkanfi et al., 2010). Pyroptosis to excess might promote sepsis by inducing immunosuppression while amplifying the inflammatory response. In this regard, activation of caspase-1 and pyroptosis in quiescent $CD4^+$ T cells was recently proposed to underlie the immunosuppressive and chronic inflammatory condition of HIV-infected individuals (Doitsh et al., 2014).

Caspase-11 responds to most intracellular Gram-negative bacteria (Broz et al., 2012; Case et al., 2013; Casson et al., 2013; Gurung et al., 2012; Rathinam et al., 2012), with those invading the cytosol being detected earlier than those remaining in vacuoles (Aachoui et al., 2013). In contrast, Gram-positive pathogens do not activate caspase-11 (Rathinam et al., 2012). The explanation for these observations is that activation of caspase-11 is triggered by acylated lipid A, a component of the LPS found in many Gram-negative bacteria (Hagar et al., 2013; Kayagaki et al., 2013). Of note, intracellular LPS or acylated lipid A activated caspase-11 in macrophages lacking the LPS receptor TLR4. Indeed, TLR4-deficient mice treated first with a TLR3 agonist to induce expression of caspase-11 were susceptible to a lethal dose of LPS, whereas the majority of caspase-11-deficient mice survived this regimen (Hagar et al., 2013; Kayagaki et al., 2013). These observations argue that there is another LPS receptor besides TLR4 whose role is to activate caspase-11 upon binding to intracellular LPS. Identification of this receptor and the substrates of the noncanonical inflammasome should shed light on the roles of human caspases 4 and 5. These caspases share highest sequence homology with mouse caspase-11, but whether they represent true functional orthologs will require further study.

Canonical Inflammasome Subtypes and Their Activation Mechanisms

Although the composition of the noncanonical inflammasome remains unknown, several canonical inflammasomes that activate caspase-1 in response to endogenous and exogenous danger signals have been characterized. Each is named after its NLR or ALR protein scaffold (Lamkanfi and Dixit, 2012). Human NLRP2, NLRP6, NLRP7, NLRP12, and the ALR protein IFI16 may assemble inflammasomes, but additional studies are needed to understand their importance for caspase-1 activation. In contrast, the role of ALR AIM2 and the NLRs NLRP1, NLRP3, and NLRC4 in inflammasome signaling is firmly established (Figure 1). In the following section, we discuss the activation mechanisms of these inflammasomes in additional detail.

The Nlrp1a and Nlrp1b Inflammasomes

Humans have a single *NLRP1* gene, whereas mice have *Nlrp1a*, *Nlrp1b*, and *Nlrp1c* genes. An important difference between human NLRP1 and its murine orthologs is that the latter lack a PYD motif at the N terminus (Boyden and Dietrich, 2006). *Nlrp1b* is highly polymorphic between mouse strains. Macrophages from 129S1 mice produce functional Nlrp1b but lack mRNA expression of the other two isoforms (Boyden and Dietrich, 2006; Sastalla et al., 2013). In contrast, C57BL/6 mice express Nlrp1a and Nlrp1c but have mutations in *Nlrp1b* that render the Nlrp1b protein nonfunctional. The role of Nlrp1c remains to be discovered, but genetic data support a role in inflammasome signaling for Nlrp1a and Nlrp1b.

Mice homozygous for an activating Q593P point mutation in Nlrp1a succumb to a systemic neutrophilic inflammatory disease at 3–5 months of age (Masters et al., 2012). Neutrophilia is caused by excessive IL-1β production and pyroptosis of hematopoietic progenitor cells. The mice exhibit profound cytopenia after chemotherapy or infection with lymphocytic choriomeningitis virus (LCMV). Biochemical studies may clarify what triggers Nlrp1a activation and why the Q593P mutation in its function-to-find (FIIND) domain renders the protein constitutively active.

The Nlrp1b inflammasome is an important defense mechanism against *Bacillus anthracis* because defective activation of the Nlrp1b inflammasome hampers host defense in mice infected with live *B. anthracis* spores (Moayeri et al., 2010; Terra et al., 2010). *Nlrp1b* is the key locus determining whether macrophages undergo pyroptosis in response to *B. anthracis* lethal toxin (LeTx) (Boyden and Dietrich, 2006). Thus, macrophages from Nlrp1b-deficient mice fail to activate caspase-1 and are defective at IL-1β secretion and pyroptosis in response to LeTx (Kovarova et al., 2012). Nlrp1b recruits caspase-1 directly

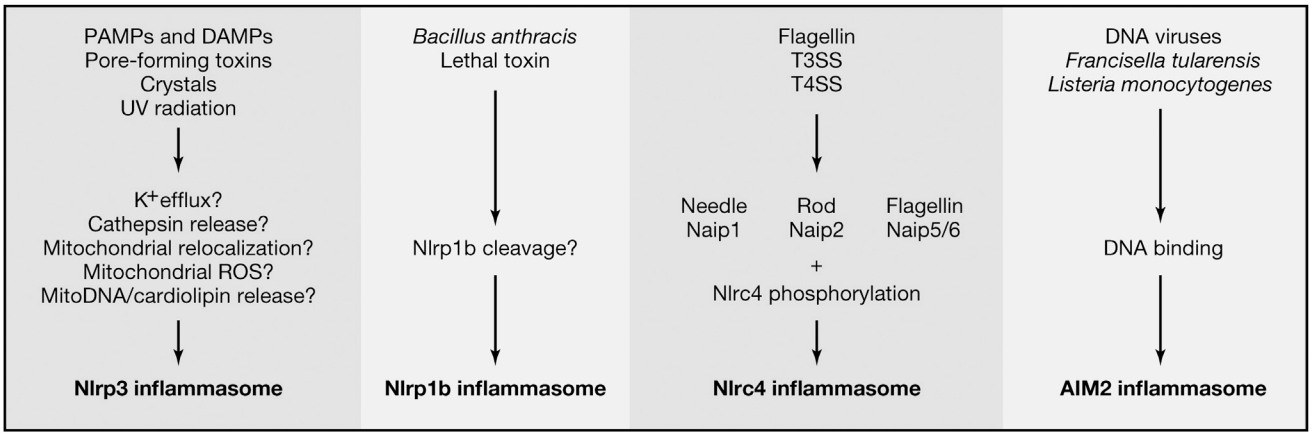

Figure 3. Schematic Overview of Proposed Inflammasome Activation Mechanisms

The different inflammasomes recruit and activate caspase-1 in response to a variety of triggers. PAMPs, DAMPs, pore-forming toxins, crystals, and UV radiation are thought to activate the Nlrp3 inflammasome by reducing intracellular K^+ concentrations, by promoting cytosolic release of lysosomal cathepsins, by relocating Nlrp3 to the mitochondrial outer membrane, and by inducing mitochondrial damage, which may be sensed by Nlrp3 via the production of ROS or the cytosolic release of oxidized mitochondrial DNA and cardiolipin. The presence of *Bacillus anthracis* lethal toxin in the cytosol might be detected through the cleavage of Nlrp1b. Cells exposed to bacteria expressing flagellin or a type III (T3SS) or IV (T4SS) secretion system indirectly activate the Nlrc4 inflammasome through Naip proteins. Mouse Naip1 and human NAIP bind the T3SS needle, while mouse Naip2 detects the rod component of T3SS and T4SS. Finally, mouse Naip5 and -6 detect bacterial flagellin in the cytosol. These bacterial factors also induce PKCδ-mediated phosphorylation of Nlrc4 on Ser533, which is required for activation of the Nlrc4 inflammasome. AIM2 is activated by the presence of dsDNA in the cytosol of cells infected with *Francisella tularensis*, *Listeria monocytogenes*, and the DNA viruses cytomegalovirus and vaccinia virus.

via its CARD motif, although the bipartite PYD-CARD adaptor protein ASC may stabilize these interactions. Indeed, ASC is critical for Nlrp1b-induced caspase-1 autoprocessing but dispensable for LeTx-induced pyroptosis and IL-1β secretion (Van Opdenbosch et al., 2014).

LeTx is a two-component toxin in which the "protective antigen" subunit provides the metalloprotease effector subunit "lethal factor" (LF) access to the cytosol. Initially, Nlrp1b was assumed to just physically associate with cytosolic LF, but it was then shown that LF metalloprotease activity was needed for its recognition (Fink et al., 2008). Subsequent studies showed that LeTx cleaves Nlrp1b close to its N terminus (Chavarría-Smith and Vance, 2013; Hellmich et al., 2012). How removal of a short (4 kDa) peptide from the N terminus promotes inflammasome activation requires further analysis (Figure 3).

The Nlrc4 Inflammasome

Similar to Nlrp1b, Nlrc4 contains a CARD motif through which it interacts with caspase-1, and this probably explains why ASC is dispensable for Nlrc4-induced pyroptosis (Figure 1). Nevertheless, ASC may amplify Nlrc4 inflammasome activity because it is critical for Nlrc4-induced caspase-1 autoprocessing and secretion of mature IL-1β and IL-18 (Broz et al., 2010b; Mariathasan et al., 2004; Van Opdenbosch et al., 2014). Nlrc4 responds to two critical components of pathogenic bacteria: flagellin, the building block of their locomotion machinery, and proteins from the type III and IV bacterial secretion systems that inject virulence factors into the host cell (Amer et al., 2006; Franchi et al., 2006; Miao et al., 2006; Miao et al., 2010b). Consequently, the Nlrc4 inflammasome is a major component of host defense against facultative intracellular pathogens such as *Salmonella* Typhimurium, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Burkholderia thailandensis*, and *Legionella pneumophila* (Lamkanfi and Dixit, 2012). Activation of Nlrc4 involves members of the Naip subfamily of NLRs (Kofoed and Vance, 2011; Rayamajhi et al., 2013; Yang et al., 2013; Zhao et al., 2011). Naip proteins have BIR motifs at their N terminus, whereas most NLRs carry a CARD or PYD motif (Lamkanfi and Dixit, 2012). The murine *Naip* locus is highly polymorphic. C57BL/6J mice express four Naip proteins: Naip1 binds to the needle of the type III secretion system (Rayamajhi et al., 2013; Yang et al., 2013), Naip2 binds to the basal rod component (Kofoed and Vance, 2011; Zhao et al., 2011), and Naip5 and Naip6 recognize flagellin (Kofoed and Vance, 2011; Zhao et al., 2011). Humans express only one NAIP homolog, and it binds to the needle structure of the type III secretion system (Yang et al., 2013; Zhao et al., 2011). Once Naip proteins have bound their ligands, they may bind to Nlrc4 to promote activation of caspase-1. Posttranslational modification of Nlrc4 also contributes to inflammasome activation, with Ser^{533} in Nlrc4 being phosphorylated after infection with *S.* Typhimurium (Qu et al., 2012). This phosphorylation site appears conserved through evolution because a mouse Nlrc4 truncation mutant lacking its CARD and an internal peptide was phosphorylated at Ser^{533} when it was expressed in insect cells (Hu et al., 2013). In crystals, CARD-less Nlrc4 adopted a solenoid shape in which the LRRs folded back onto the NACHT domain (Hu et al., 2013). Further investigation of the relationship between Naip detection of bacterial components and Nlrc4 phosphorylation may illuminate how this inflammasome is activated.

The Nlrp3 Inflammasome

The Nlrp3 inflammasome is assembled when the amino-terminal PYD of Nlrp3 engages in homotypic interactions with the PYD of ASC to recruit caspase-1. However, the mechanisms associated with activation of the Nlrp3 inflammasome continue to be debated. This inflammasome is activated by bacterial, viral,

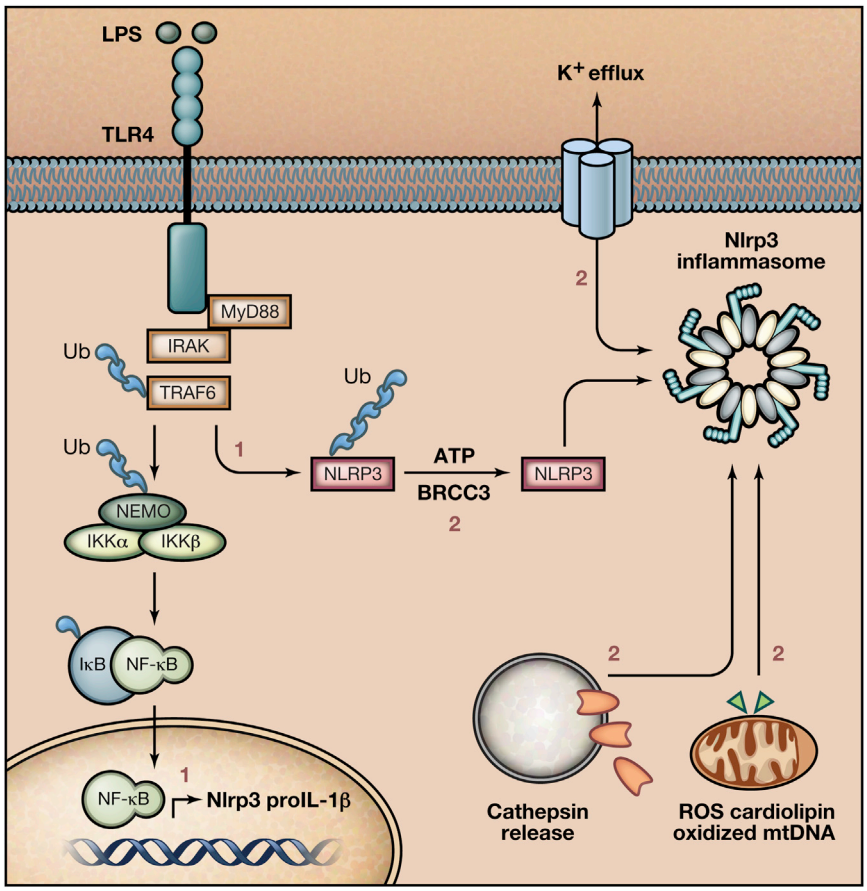

Figure 4. Priming and Activation Signals of the Nlrp3 Inflammasome

Although the exact molecular mechanisms of NLRP3 inflammasome assembly are incompletely understood, it is well-established that Nlrp3 activation requires two signals. Binding of the TLR4 ligand LPS to its receptor provides the first signal by triggering NF-κB-mediated upregulation of Nlrp3 along with proIL-1β. Alternatively, TLR4 may provide signal 1 through an incompletely understood pathway involving its adaptor molecules MyD88, IRAK1, and IRAK4 without the need for new protein synthesis. BRCC3-mediated K63-deubiquitination of Nlrp3 is required for Nlrp3 inflammasome assembly and activation by extracellular ATP and other Nlrp3-activating stimuli. These agents provide a second signal in the form of K^+ efflux, cytosolic release of lysosomal cathepsins, relocalization of Nlrp3 from the cytosol to mitochondria, or cytosolic release of mitochondria-derived factors such as reactive oxygen species (ROS), cardiolipin, and oxidized mitochondrial DNA (mtDNA).

and fungal pathogens, pore-forming toxins, crystals, aggregates such as β-amyloid, and DAMPs such as ATP and hyaluronan (Lamkanfi and Dixit, 2012). It is generally agreed that detection of such a diversity of agents cannot be direct. Instead, Nlrp3 is thought to monitor some host-derived factor that is altered by these agents. Several hypotheses for Nlrp3 activation have been formulated. They can be summarized as follows: Nlrp3 is activated by: (1) K^+ efflux (Muñoz-Planillo et al., 2013), (2) translocation to mitochondria (Misawa et al., 2013; Subramanian et al., 2013; Zhou et al., 2011), (3) the production of mitochondrial reactive oxygen species (Zhou et al., 2011), (4) the release of mitochondrial DNA or the mitochondrial phospholipid cardiolipin (Iyer et al., 2013; Nakahira et al., 2011; Shimada et al., 2012), and (5) the cytosolic release of lysosomal cathepsins (Hornung et al., 2008). A single unifying event has not emerged because some of these events do not occur with all Nlrp3-activating agents, or they are associated with multiple inflammasomes, or their occurrence is contested (Bauernfeind et al., 2011; Muñoz-Planillo et al., 2013; Pétrilli et al., 2007). There is disagreement too as to whether PKR (Lu et al., 2012), TXNIP (Zhou et al., 2010), and MAVS (Subramanian et al., 2013) have important roles in the Nlrp3 pathway (Ermler et al., 2014; He et al., 2013; Masters et al., 2010; Muñoz-Planillo et al., 2013).

What is clear is that activation of the Nlrp3 inflammasome requires two signals (Figure 4). First, an NF-κB-activating stimulus is required for cells to express pro-IL-1β and optimal Nlrp3 (Bauernfeind et al., 2009). Actual assembly of the Nlrp3 inflammasome occurs when a second signal is provided by an Nlrp3-activating agent (Lamkanfi and Dixit, 2012). Although Nlrp3 induction certainly contributes to inflammasome activation, brief TLR stimulation that doesn't increase the amount of Nlrp3 is sufficient to prime the pathway for activation by ATP (Juliana et al., 2012; Schroder et al., 2012). This alternative signal 1 is provided by TLR4 and is relayed by its adaptors MyD88, IRAK1, and IRAK4 independently of new protein synthesis (Fernandes-Alnemri et al., 2013; Juliana et al., 2012; Lin et al., 2014). Intriguingly, Nlrp3 deubiquitination is required for inflammasome assembly and activation (signal 2) (Juliana et al., 2012; Lopez-Castejon et al., 2013; Py et al., 2013). The K63-specific deubiquitinase BRCC3 was proposed to remove ubiquitin chains from the Nlrp3 leucine-rich repeat (LRR) motifs at this step (Py et al., 2013). Whether this triggers Nlrp3 relocalization, induces conformational changes, or serves other purposes that promote Nlrp3 inflammasome assembly is not known. Also the identity of the E3 ubiquitin ligase that modifies Nlrp3 in the basal state is unclear.

The AIM2 Inflammasome

The ALR protein AIM2 assembles a canonical inflammasome that recruits ASC to activate caspase-1. It does so when its DNA-binding HIN200 domain detects DNA from intracellular pathogens such as *Francisella tularensis*, cytomegalovirus, and vaccinia virus (Alnemri, 2010; Kanneganti, 2010). Mice lacking AIM2 or caspase-1 fail to clear infections with *F. tularensis*, the causative agent of tularaemia, illustrating the critical role that the AIM2 inflammasome plays in host defense against microbial pathogens (Alnemri, 2010). In association with Nlrp3 and Nlrc4, AIM2 also contributes to caspase-1 activation by *Listeria monocytogenes* (Rathinam et al., 2010; Sauer et al., 2010; Wu et al.,

2010). It was suggested that AIM2 may be linked to the pathology of autoimmune disorders such as systemic lupus erythematosus where DNA-autoantibodies are abundant (Zhang et al., 2013). If proven true, the pathway becomes a candidate for targeted therapies.

Of note, AIM2 inflammasome activation in macrophages coincides with the formation of a single large perinuclear aggregate or "speck" (Jones et al., 2010). Specks are also seen upon activation of the Nlrp1b, Nlrc4, and Nlrp3 inflammasomes (Broz et al., 2010a; Broz et al., 2010b; Van Opdenbosch et al., 2014). Speck formation is explained by the tendency of the ASC PYD domain to polymerize into star-shaped, branched filaments that serve as platforms for caspase-1 clustering (Cai et al., 2014; Lu et al., 2014). AIM2 and Nlrp3 nucleate ASC fibers through their PYD domains (Cai et al., 2014; Lu et al., 2014). The CARD of Nlrc4 triggers formation of similar ASC fibers, and again, the PYD of ASC is important (Cai et al., 2014). Of note, however, speck formation is not an absolute requirement for inflammasome activity because *S.* Typhimurium activates caspase-1 and induces pyroptosis even in the absence of ASC (Broz et al., 2010b; Van Opdenbosch et al., 2014). Nlrp1b-induced pyroptosis and IL-1β secretion also have been reported to occur in the absence of ASC (Van Opdenbosch et al., 2014).

Therapeutic Potential of Inflammasome Modulation

Aberrant inflammasome signaling contributes to pathology in a large number of infectious (Lamkanfi and Dixit, 2011) and autoimmune diseases (Lamkanfi and Dixit, 2012; Strowig et al., 2012). Recent work implicated inappropriate inflammasome signaling in graft-versus-host disease (Jankovic et al., 2013), type 2 diabetes (Jourdan et al., 2013; Masters et al., 2010), obesity-induced asthma (Kim et al., 2014), and insulin resistance (Stienstra et al., 2011; Vandanmagsar et al., 2011; Wen et al., 2011). Moreover, the NLRP3 inflammasome is activated during age-related macular degeneration (AMD) (Marneros, 2013; Tarallo et al., 2012). The accumulation of drusen and retinal damage in AMD is the leading cause of central vision loss in the elderly. Nlrp3 inflammasome blockade augmented retinal damage in an acute laser-induced wound-healing model (Doyle et al., 2012), but chronic NLRP3-driven IL-1β production may contribute to AMD-associated chorioretinal pathology in patients. This hypothesis is supported by studies demonstrating that deletion of Nlrp3 and caspase-1 reduced AMD pathology in two mouse models that resemble age-dependent aspects of human AMD (Marneros, 2013; Tarallo et al., 2012).

Alzheimer's disease is another age-related degenerative disorder that is exacerbated by NLRP3 inflammasome activity. Fibrillar β-amyloid deposits engage the NLRP3 inflammasome in cultured microglia and recruit activated microglia to β-amyloid plaques in the brain in vivo (Halle et al., 2008). More recently, deletion of Nlrp3 or caspase-1 was demonstrated to reduce memory loss and the accumulation of chronic β-amyloid deposits in transgenic mouse models of Alzheimer's disease (Heneka et al., 2013). In keeping with these observations, microglia in the vicinity of β-amyloid plaques in either Alzheimer's patients or mouse models of the disease were shown to express more IL-1β (Kim and de Vellis, 2005; Simard et al., 2006). In addition, mice lacking IL-1 receptor antagonist (IL-1Ra) exhibited more neuronal damage when exposed to exogenous β-amyloid (Craft et al., 2005).

Anti-IL-1 therapies have proven successful in several of the aforementioned ailments, including type 2 diabetes and juvenile rheumatoid arthritis (Dinarello et al., 2012; Larsen et al., 2007). Clinical studies may establish the therapeutic validity of inflammasome inhibition in additional inflammatory disorders in the future. To highlight a number of possible therapeutic approaches, we will elaborate on inflammasome blockade in autoinflammatory syndromes, which are often associated with mutations in inflammasome-related genes.

Inherited autoinflammatory diseases are characterized by recurrent episodes of inflammation in the absence of high-titer autoantibodies and antigen-specific T cells. These criteria differentiate them from autoimmune disorders in which autoreactive antibodies and lymphocytes play a central role in pathogenesis. Several autoinflammatory disorders are caused by mutations in genes mediating or modulating inflammasome activation. Well-studied examples are the cryopyrin-associated periodic fever syndromes (CAPS), which include familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and chronic infantile neurological cutaneous and articular syndrome/neonatal onset multisystem inflammatory disease (CINCA/NOMID) (Feldmann et al., 2002; Hoffman et al., 2001). These diseases produce urticarial skin rashes and prolonged episodes of fever, and a significant number of patients have gain-of-function mutations in *NLRP3* (Feldmann et al., 2002; Hoffman et al., 2001). The CAPS-associated mutations probably produce conformational changes in NLRP3 that render the protein constitutively active, thereby causing persistent caspase-1 activation and disproportionate production of IL-1β and IL-18 (Dowds et al., 2004).

Inhibition of IL-1 signaling has proven remarkably beneficial in CAPS patients (Ter Haar et al., 2013). Several other autoinflammatory disorders, including tumor necrosis factor receptor-associated periodic syndrome (TRAPS), in which disease is causally linked with mutations in TNF-R1, have also benefited from anti-IL-1 therapy. In fact, TRAPS patients respond significantly better to IL-1 inhibition than to TNF blockade (Ter Haar et al., 2013). Anti-IL-1 molecules currently approved for these syndromes include rilonacept (Hoffman et al., 2012), anakinra (Goldbach-Mansky et al., 2006), and canakinumab (Kuemmerle-Deschner et al., 2011). Anakinra is a non-glycosylated human IL-1 receptor antagonist, and rilonacept contains the ligand-binding domains of human IL-1 receptor (IL-1R1) and IL-1 receptor accessory protein (IL-1RAcP) fused to the Fc portion of a human immunoglobulin G1 (IgG1). The interaction of anakinra and rilonacept with the IL-1 receptor prevents IL-1α and IL-1β from binding to the IL-1 receptor and exerting their biological functions. In contrast, canakinumab is a humanized monoclonal antibody that neutralizes IL-1β in circulation and does not interfere with binding of IL-1α to the IL-1 receptor. Several other molecules targeting either IL-1β or IL-1R are currently under development (Dinarello et al., 2012).

Despite their remarkable efficacy, anti-IL-1 therapies do not resolve all CAPS-associated symptoms (Neven et al., 2010). One possible explanation is that the caspase-1-dependent

cytokine IL-18 still promotes disease. In mice expressing CAPS-associated *Nlrp3* mutations, IL-18 was important early in disease development, whereas the effects of IL-1 dominated at later stages (Brydges et al., 2013). Of note, however, a number of CAPS mice lacking both IL-18 and the IL-1 receptor still succumbed to disease, whereas caspase-1 deficiency provided full protection. Therefore, other caspase-1-mediated pathways, such as pyroptosis, may contribute to CAPS pathology. These findings highlight the potential benefits of blocking the NLRP3 inflammasome directly over inhibiting its downstream cytokines. It is possible that CAPS and other patients might benefit from pharmacologic inhibitors of caspase-1 such as VX-765, which is presently being tested in epilepsy. This compound prevented IL-1β secretion from LPS-stimulated peripheral blood mononuclear cells of FCAS patients in vitro (Stack et al., 2005), suggesting therapeutic potential in CAPS disease. Continued development of this and other caspase-1 inhibitors could potentially offer patients with autoinflammatory and related immune disorders additional options for improving their quality of life.

Conclusion and Perspectives

This Review has provided a discussion of the key advances that have been made in understanding the roles and activation mechanisms of inflammasomes and illustrated their increasingly appreciated roles in infectious, autoimmune, and autoinflammatory diseases. The existence of a noncanonical inflammasome that responds to intracellular LPS and Gram-negative bacteria has been proposed, the importance of posttranslational modifications in the activation of Nlrc4 and Nlrp1b demonstrated, and the contribution of pyroptosis in host defense against microbes and its role in autoinflammation clarified. These recent developments have raised some fascinating new questions for the field nonetheless. For instance, how does deubiquitination modulate Nlrp3 activation, what is the identity of the LPS sensor activating caspase-11, and how do caspases 1 and 11 trigger pyroptosis?

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